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Original article

Characterization of class 1, 2, 3 integrons, ESBL genes and antibiotic susceptibility of *Salmonella* serotypes from broiler and cattles in Turkey

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Abstract

Antimicrobial resistance in *Salmonella* has been associated with the presence of integrons and many other resistance mechanisms contributing to the spread of antimicrobial-resistant genes within and between livestock and human populations. In this study, the presence of *Salmonella* serovars from broiler and cattle samples and their antimicrobial resistance, integrons, *tet* resistance, ESBL and resistance genes carriage were investigated. Total of 209 litter (broiler farms) and fecal samples (cattle farms) were examined by bacteriological procedures, susceptibilities against 18 antimicrobials and genes carriages were detected by singleplex and multiplex PCR. A total of 46/209 (22 %) *Salmonella* strains were isolated. Six different *Salmonella* serotypes from 46 *Salmonella* isolates were identified and the most common serotype was *S.* Infantis 38 (82.6%) from broiler litter; followed by *S.* Kitenge 3 (6.5 %) from fecal sample. The highest occurrence of resistance observed for penicilline (46/46, %100), lincomycin (43/46, 93.5%) and 42 isolates (43/46, 93.5%) exhibited MDR. The overall occurrence of class 1, 2 and 3 integrons carrying *Salmonella* in tested samples were 63.04% (29/46), 43.5% (20/46) and 84.8% (39/46) respectively. Out of the 27 isolates produced an ESBL, mostly CTX and TEM. On 46 *Salmonella* isolates, in 16 (34.8%) Tcr' genes were determined. Genotypic and phenotipic detection of ESBL genes found within integrons from *Salmonella* isolates from different sources (broiler and cattle) can provide powerful information about health and economic risk associated with transferable multidrug resistance.

Key words: antibiotic susceptibility, broiler, cattle, integrons, β-lactamase, MARI, Salmonella, tet genes

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Introduction

Salmonella, which has a wide range of hosts from cold-blooded animals to humans, is the causative agent of the most common foodborne diseases in the world. Some serotypes show host-specific characteristics, but the vast majority cause cross-species infections; hence, warm-blooded animals-origin *Salmonella* serotypes are considered potential pathogens for humans (Akiba et al. 2010).

Monitoring of emerging and existing antimicrobial resistance is a major public health concern and understanding the development and spread of resistance is to identify the risks and deal with resistance by taking target measures (EFSA 2021). Antibiotic resistance in *Salmonella* have emerged from misuse of antibiotics such as growth promoters, their excessive use in clinical treatments of disease, and also other causes should also be considered (Roca et al. 2015, Zwe et al. 2018). Several common *Salmonella* serovars are more resistant to antimicrobials than others (Thomas et al. 2020). The most recent example of this situation is the worldwide spread of the MDR *S.* Typhimurium phage type DT104 in humans and animals. The highlighted factor is that DT104 is resistant to ampicillin, chloramphenicol, streptomycin, sulfanamide and tetracyclines (ACSSuT resistant type) (Threlfall 2000, 2002). Recently, many serotypes of *Salmonella* spp. started to show resistance to antibiotics such as quinolones, cephalosporin, β-lactam family, aminoglycosides, tetracyclines, and etc. (Castro-Vargas et al. 2020).

Although some researchers also argue that there are still gaps in our knowledge on resistance spread from animals to environment, or vice versa (Chang et al. 2015), antimicrobial resistance can be spread through food as well as water, environmental contamination and direct animal contact (Pan et al. 2019, Paudyal et al. 2019). With the increased number of multidrug-resistant (MDR) strains all over the world, attempts for the monitoring, control and treatment of *Salmonella* have gained momentum (Duc et al. 2019, Thomas et al. 2020).

Antimicrobial resistance in *Salmonella* has been associated with the presence of integrons and especially one to three smaller mobile elements (gene casettes) have been identified in various integron classes. Integrons are divided into 1-5 classes that contain integrase gene and in previous studies were presented in particular class 1 and class 2 integrons, which have been shown to harbour resistance genes to many classes of antimicrobials (Rao et al. 2008, Barlow et al. 2009, Firoozeh et al. 2011, Ahmed and Shimamoto 2014). In recent years, although prevalence of integrons in food-borne MDR *Salmonella* isolates is increasing all over the world, there are limited data on the presence of class 3 integrons (Asgharpour et al. 2018).

Beta-lactamases produced in Gram negative bacteria are enzymes synthesized via chromosomes, plasmids or transposons which cleave the amide bonds in beta-lactam antibiotics (Yusuf et al. 2021), and today there are more than 200 extended-spectrum beta-lactamases (ESBL) such as TEM-, SHV-, OXA- and CTX-M and the number of ESBL-producing bacteria has increased worldwide in many different genera of *Enterobacteriaceae* (Bush and Jacoby 2010, de Jong et al. 2014). Previous data suggested that the most common ESBLs were the TEM-, however over the last years were faced with the CTX-M types increasing (Paterson and Bonomo 2005, Livermore et al. 2006).

The aim of this work was to investigate the prevalence of *Salmonella* serovars from broiler and cattle samples, as well as detecting the genetic determinants responsible for (ESBL) resistance, integrons and tetracycline resistance (Tcrs).

Materials and Methods

Sample Collection, *Salmonella* Isolation and Identification

Eighty litter samples were collected randomly from 27-38 day-old broilers in 80 broiler houses (11000--90000 poultry capacity) and one hundred twenty nine fresh fecal samples were collected randomly on the ground (immediately after defecation) from 0.5-2 years aged cattles, (100-250 cattle capacity) in 15 cattle farm.

All litter and fecal samples were analyzed for *Salmonella* according to ISO 6579-1:2017 (ISO 2017). Samples were inoculated in buffered peptone water (BPW) as pre-enrichment medium and incubated at 37°C for 18-24 h. After incubation, samples were transferred to Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn) and modified semi-solid Rappaport-Vassiliadis (MSRV) medium and enriched for 18-24 h at 37°C and 24 h at 41.5°C, respectively. The cultures obtained were plated onto xylose lysine deoxycholate (XLD). All presumptive *Salmonella* colonies were characterized biochemically (triple sugar iron (TSI), H_2S, gas formation, voges proskauer (VP), urea, lysine decarboxylase, and β-galactosidase tests) by Microgen® GN-ID A sytem (Microgen Bioproducts, UK) (ISO 2017, Issenhut-Jeanjean et al. 2014).

The serotyping of the strains that are biochemically compatible with *Salmonella* spp. were made by slide agglutination using polyvalent and monovalent *Salmonella* "O" and "H" antisera and serotyped according to the Kauffman-White scheme (Le Minor 1992)

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Antimicrobial susceptibility testing

Antimicrobial susceptibility test was carried out by the agar disk diffusion method according to the guidelines from Clinical and Laboratory Standards Institute on Mueller-Hinton agar (Oxoid Ltd, Hampshire, UK) according to the guidelines from Clinical and Laboratory Standards Institute (CLSI 2020). The following antibiotics were selected: ampicillin (10 µg; AMP), amoxicillin-clavulanic acid (25µg; AMC), amoxicillin (25 µg; AX), cefixime (30 µg; CXM), cefotaxime (5 µg; CTX), ceftazidime (30 µg; CAZ), cefoxitime (30µg; FOX), ceftiofur (5 µg; FUR), cephalothin (5 µg; KF), colistin sulphate (10 µg; CT), enrofloxacin (5 µg; ENR), gentamicin (10 µg; CN), florfenicol (30 µg; FFC), lincomycin (15 µg; MY), nalidixic acid (30 µg; NA), neomycin (30 µg; N), doxycilin (30 µg; DO), oxytetracycline (30 µg; OT), tetracycline (10 µg; T), penicillin (10 units; P), sulphamethoxazole trimethoprim (25 µg; SXT), streptomicin (10 µg; S), piperacillin/tazobactam (36 µg; TZP). The results were obtained by measuring the diameter of the growth inhibition zone around the antibiotic disc for each isolated bacterial strain and recorded as sensitive, intermediate and resistant. Isolates displaying resistance to ≥3 antimicrobials tested were defined as exhibiting multidrug-resistance (MDR) (CLSI 2020).

Multiple antibiotic resistance index (MARI) was determined for each *Salmonella* serotype by using the formula MARI = a/b, where a represents the number of antibiotics to which the test isolate depicted resistance and b represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility (Krumperman 1983).

Statistical analysis

Descriptive statistics is shown as mean ± standard deviation. Normality assumption was checked with Shapiro Wilk Test. Mann Whitney-U Test was carried out to determine the mean difference between cattle and broiler in MAR index. Statistical analysis was performed using SPSS 23.0. $p<0.05$ was considered as statistically significance level.

Detection of Antimicrobial Genes

Primers for each PCR are listed in Table 1. DNA extractions from *Salmonella* isolates were performed according to the instructions of the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA). DNAs were stored for use as template DNA at -20°C until amplification.

PCR assay was carried out for β-lactamase, P*ampC* and integron (I, II, III) and *tet* genes (Table 1). Each PCR reaction' mix concentration and amplification conditions were carried out following the previous protocols (Goldstein et al. 2001, Ng et al. 2001, Leverstein-van Hall et al. 2003, Machado et al. 2007).

Results

Isolation and identification

A total of 46/209 (22 %) *Salmonella* strains were isolated from litter and fecal samples. Six different *Salmonella* serotypes from 46 *Salmonella* isolates were identified and the most common serotype was *S.* Infantis 38 (82.6%), from broiler litter; followed by *S.* Kitenge 3 (6.5 %) from fecal sample.

Antimicrobial susceptibility testing

The results of the antimicrobial susceptibility analysis of 46 *Salmonella* isolates are presented in Table 2. The highest occurrence of resistance observed was for penicilline (46/46), lincomycin (43/46, 93.5%), followed by cephalothin 32/46, 69.6%), streptomicin (31/46, 67.4%). In contrast, low level of resistance was found for ceftiofur (1/46, 2.2%), florfenicol (2/46, 4.4%), cefoxitime and ceftazidime (3/46, 6.5%), amoxicillin-clavulanic acid, piperacillin/tazobactam and cefotaxime (4/46, 8.7%). In addition, 42 isolates (43/46, 93.5%) exhibited MDR. The most frequent MDR pattern was lincomycin, peniciline, gentamycin, tetracycline which was represented by *S.* Enteritidis and *S.* Kitenge (2/43, 4.7%) from cattle and cephalothin, penicilline, lincomycin, streptomycin, tetracycline and neomycin which were represented by *S.* Infantis (38/43, 88.4%) from broiler. *S.* Montevideo from cattle was pansusceptible.

MAR index were revealed among 46 *Salmonella* serotypes, and to 8 (17.4%) was less and to 38 (82.6%) was greater than 0.2. However, four *Salmonella* isolates had shown MARI of 0.1 (i.e. resistant to all the antimicrobials tested), out of which two were recovered from cattles, and one from broiler (Fig. 1).

Statistical analysis

There is a statistically significant difference between cattle (0.13±0.05) and broiler (0.68±0.27) in terms of MAR index ($p<0.001$) (Fig. 2).

Table 1. ESBL, integron and tetracycline resistance genes' primer sequences for PCR assays.

Genes	Sequences (5'-3')	Amplicon size (bp)
int 1	TCTCGGGTAACATCAAGG AGGAGATCCGAAGACCTC	254
int 2	CACGGATATGCGACAAAAAGG TGTAGCAAACGAGTGACGAAATG	788
int 3	AGTGGGTGGCGAATGAGTG TGTTCTTGTATCGGCAGGTG	600
*bla*TEM	GCGGAACCCCTATTTG TCTAAAGTATATATGAGTAAACTTGGTCTGAC	964
P*ampC*	GTGAATACAGAGCCAGACGC GTTGTTTCCGGGTGATGC	343
*bla*SHV	TTCGCCTGTGTATTATCTCCCTG TTAGCGTTGCCAGTGYTCG	854
*bla*CTX	ATGTGCAGYACCAGTAARGTKATGGC TGGGTRAARTARGTSACCAGAAYCAGCGG	593
*bla*CMY-1 group	GTGGTGGATGCCAGCATCC GGTCGAGCCGGTCTTGTTGAA	915
*bla*CMY-2 group	GCACTTAGCCACCTATACGGCAG GCTTTTCAAGAATGCGCCAGG	758
*bla*OXA-1	ATGAAAAACACAATACATATCAACTTCGC GTGTGTTTAGAATGGTGATCGCATT	820
*bla*OXA-2	ACGATAGTTGTGGCAGACGAAC ATYCTGTTTGGCGTATCRATATTC	602
*bla*ACC-1	AGCCTCAGCAGCCGGTTAC GAAGCCGTTAGTTGATCCGG	818
tet(A)	GCT ACA TCC TGC TTG CCT TC CAT AGA TCG CCG TGA AGA GG	210
tet(B)	TTG GTT AGG GGC AAG TTT TG GTA ATG GGC CAA TAA CAC CG	659
tet(C)	CTT GAG AGC CTT CAA CCC AG ATG GTC GTC ATC TAC CTG CC	418
tet(D)	AAA CCA TTA CGG CAT TCT GC GAC CGG ATA CAC CAT CCA TC	787
tet(E)	AAA CCA CAT CCT CCA TAC GC AAA TAG GCC ACA ACC GTC AG	278
tet(G)	GCT CGG TGG TAT CTC TGC TC AGC AAC AGA ATC GGG AAC AC	468
tet(G)	CAG CTT TCG GAT TCT TAC GG GAT TGG TGA GGC TCG TTA GC	844
tet(K)	TCG ATA GGA ACA GCA GTA CAG CAG ATC CTA CTC CTT	169
tet(L)	TCG TTA GCG TGC TGT CAT TC GTA TCC CAC CAA TGT AGC CG	267
tet(M)	GTG GAC AAA GGT ACA ACG AG CGG TAA AGT TCG TCA CAC AC	406
tet(O)	AAC TTA GGC ATT CTG GCT CAC TCC CAC TGT TCC ATA TCG TCA	515
tet(S)	CAT AGA CAA GCC GTT GAC C ATG TTT TTG GAA CGC CAG AG	667
tet(P)	CTT GGA TTG CGG AAG AAG AG ATA TGC CCA TTT AAC CAC GC	676
tet(Q)	TTA TAC TTC CTC CGG CAT CG ATC GGT TCG AGA ATG TCC AC	904
tet(X)	CAA TAA TTG GTG GTG GAC CC TTC TTA CCT TGG ACA TCC CG	468

Table 2. Antimicrobial resistance profiles, ESBLs, integrons and resistance genes of *Salmonella* serotypes isolates from cattle and broilers.

		Serovar	Antimicrobial resistance	Integrons/Resistance genes
Cattle	1	*S.* Montevideo	MY, P	*int1, int3*, *tet* B
	2	*S.* Enteritidis	MY,P, CN, TE	*int1*, bla_{CTX}
	3	*S.* Enteritidis	MY,P, CN, TE	*int1*
	4	*S.* Kitenge	MY,P	*int1*
	5	*S.* Kitenge	MY,P,TE	*int1, tet* B
	6	*S.* Kitenge	MY,P,TE	*int1*
Broiler	7	*S.* Infantis	KF, AMP, AMC, AX, MY, P, S	*int2*, *int3,* bla_{CTX}
	8	*S.* Infantis	KF, SXT, T, MY, DO, NA, P, TE, S	*int3,* bla_{CTX}, bla_{TEM}
	9	*S.* Infantis	KF, SXT, T, MY, DO, NA, P, TE, S	*int2*, *int3*, *tet* D, bla_{OXA-2}
	10	*S.* Infantis	KF, T, MY, DO, NA, P, TE, S	*int1, int3,* $bla_{CMY-1\ group}$
	11	*S.* Infantis	SXT,T, MY, DO, P, TE, S, N	*int1, int2, int3*, *tet* B, *tet* D, bla_{CTX}
	12	*S.* Infantis	CXM, CTX, KF, T, MY, CT, DO, NA, ENR, P, TE, S	*int3,* bla_{CTX}, bla_{TEM}
	13	*S.* Infantis	CXM, CTX, KF, SXT, T, MY, DO, NA, ENR, P, S, TE, CT	*int1, int2, int3, tet* M, bla_{CTX}
	14	*S.* Infantis	KF, SXT, T, MY, DO, NA, P, TE, S	*int1, int2, int3*, *tet* B
	15	*S.* Infantis	KF, SXT, T, MY, DO, NA, P, TE, S, N	*int1, int2, int3,* bla_{CTX}
	16	*S.* Infantis	KF, T, MY, DO,NA, P, TE, S	*int1, int2, int3,* bla_{TEM}
	17	*S.* Infantis	KF, SXT, T, MY, DO, NA, P, TE, S	*int1, int3*
	18	*S.* Infantis	KF, AMP, AX, SXT, MY, P, S, N, TZP	*int1, int3* $bla_{CMY-2\ group}$, bla_{TEM}
	19	*S.* Infantis	KF, SXT, T, MY, DO, NA, P, TE, S, N	*int1, int3,* bla_{ACC-1}
	20	*S.* Infantis	KF, SXT, T, MY, NA, P, TE, S, N, CT	*int1, int2, int3, tet* B, bla_{OXA-1}
	21	*S.* Infantis	KF, AMP, AMC, AX, CN, MY, P, TE, S, N	*int1, int2, int3*, *tet* B
	22	*S.* Infantis	KF, SXT, T, MY, DO, NA, P, TE, S, N	*int3* $bla_{CMY-2\ group}$
	23	*S.* Infantis	KF, T, MY, DO, NA, P, TE, S, TZP	*int3*
	24	*S.* Infantis	SXT, T, MY, DO, P, TE, S, N	*int1, int3, tet* B, bla_{OXA-2}
	25	*S.* Infantis	CXM, CTX, KF, T, MY, DO, NA, P, TE, S, N	*int1, int2, int3,* bla_{CTX}
	26	*S.* Infantis	CXM, FOX, CAZ, KF, SXT, T, MY, DO, NA, ENR, P, TE, S, N	*int1, int2, int3* $bla_{CMY-1\ group}$, bla_{CTX}
	27	*S.* Infantis	KF, SXT, T, CN, MY, DO, FFC, NA, P, ENR, TE, S, N	*int2,* int3, bla_{SHV}, bla_{OXA-2}, bla_{CTX}
	28	*S.* Infantis	KF, SXT, T, MY, DO, CT, NA, ENR, P, TE, S, N	*int2, int3*, bla_{OXA-1}, *tet* B, bla_{OXA-2}
	29	*S.* Infantis	FUR, KF, AMP, AMC, T, MY, DO, NA, P, TE, S, N	*int1, int3,* bla_{ACC-1}
	30	*S.* Infantis	KF, AX, ST, T, MY, DO, TZP, NA, P, TE, S	*int1, int3, tet* M
	31	*S.* Infantis	KF, AMP, AX, SXT, MY, P, S, N	*int3,* bla_{SHV}
	32	*S.* Infantis	KF, SXT, T, MY, DO, NA, P, TE, S, N	*int1, int2, int3,* bla_{TEM}
	33	*S.* Infantis	KF, SXT, T, MY, NA, P, TE, S, N	*int1, int2, int3,* bla_{TEM}
	34	*S.* Infantis	KF, AMP, AMC, AX, CN, MY, TZP, P, TE, N, S	*int1, int2, int3,* bla_{ACC-1}
	35	*S.* Infantis	KF, SXT, T, MY, DO, NA, P, TE, N	*int2, int3, tet G*
	36	*S.* Infantis	KF, T, MY, DO, NA, P, TE, S, N	*int1, int2, int3*
	37	*S.* Infantis	T, CN, MY, DO, P, TE	*int1, int3*
	38	*S.* Infantis	CXM, KF, T, MY, DO, FFC, NA, ENR, P, TE, N, S	*int1, int3,* $bla_{CMY-1\ group}$
	39	*S.* Infantis	CXM, CTX, CAZ, T, P	*int1, int3, tet* B, *tet* D
	40	*S.* Infantis	T, CT, ENR, P	*int1, int3, tet* B
	41	*S.* Infantis	MY, T, P, TE	*int1, int3,* bla_{TEM}
	42	*S.* Infantis	KF, T, CN, P	*int1, int2, int3*
	43	*S.* Infantis	KF, SXT, T, MY, DO,CT, NA, ENR, P, TE, S, N	*int1, int2, int3*
	44	*S.* Infantis	MY, P	*int3*
	45	*S.* Mbandaka	CXM, FOX, CAZ, KF, MY, DO, SXT, T, NA, P, TE	bla_{ACC-1}
	46	*S.*Typhimurium	FOX, MY, P, CN, ENR, P	*int1*, $bla_{TEM,}$ bla_{ACC-1}

Ampicillin (10 µg; AMP), Amoxicillin clavulanic acid (25 µg; AMC), Amoxicillin (25 µg; AX), Cefixime (30 µg; CXM), Cefotaxime (5 µg; CTX), Ceftazidime (30 µg; CAZ), Cefoxitime (30 µg; FOX), Ceftiofur (5 µg; FUR), Cephalothin (5 µg; KF), Colistin sulphate (10 µg; CT), Enrofloxacin (5 µg; ENR), Gentamicin (10 µg; CN), Florfenicol (30 µg; FFC), Lincomycin (15 µg; MY), Nalidixic acid (30 µg; NA), Neomycin (30 µg; N), Doxycilin (30 µg; DO), Oxytetracycline (30 µg; T), Tetracycline (10 µg; TE), Penicillin (10 units; P), Sulphamethoxazole Trimethoprim (25 µg; SXT), Streptomicin (10 µg; S), Piperacillin/Tazobactam (36 µg; TZP).

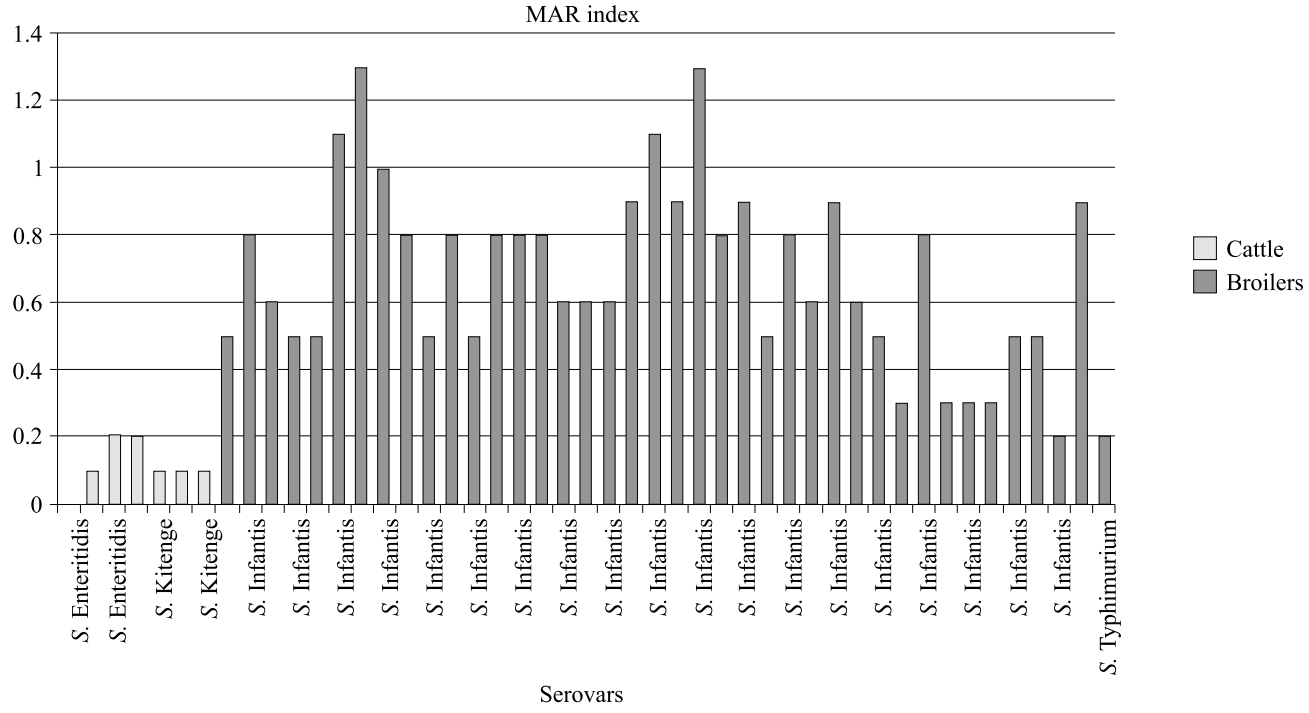

Fig. 1. Diagram of MAR index results.

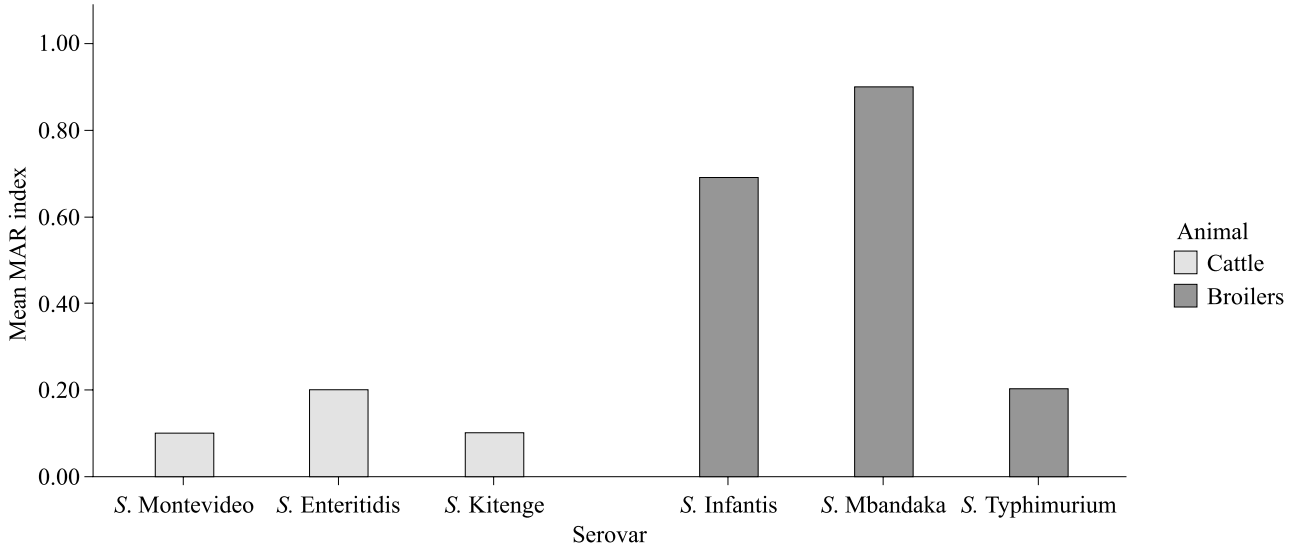

Fig. 2. Statistical analysis of MAR index.

Detection of antimicrobial genes

PCR analysis for antimicrobial resistance revealed that 4 β-lactamase genes were detected among the isolates, and carried bla_{CTX} (10/46, 21.8%), followed by bla_{TEM} (8/46, 17.4%), bla_{ACC-1} (5/46, 10.9%), bla_{OXA-2} (4/46, 4.4%), $bla_{CMY-1group}$ (3/46, 6.52%), bla_{SHV} and $bla_{CMY-2group}$ (2/46, 4.34%), bla_{OXA-1} (1/46, 2.2%). No isolates expressed the P*ampC* gene. Of the 46 *Salmonella* isolates, 15 (32.6%) Tcr' genes were determined; 10 (35%) *tet* (B), 3(10%) *tet*(D), 2(10%) *tet* (M), 1 (2.2%) and two isolates were found to carry both *tet* (B) and *tet* (D).

Detection of integrons

The overall occurrence of class 1, 2 and 3 integrons carrying *Salmonella* in tested samples was 63.04% (29/46), 43.5% (20/46) and 84.8% (39/46), respectively. *S.*Mbandaka, *S.* Montevideo, *S.* Enteritidis (one) and *S.* Kitenge were not carrying any of the three integrons, *S.* Infantis was the most prevalent serovar in both three class 1 integrons-carrying and all *S.* Infantis were carrying integron 3.

Discussion

The emergence of antimicrobial resistance in zoonotic bacteria has major public health implications. Data suggest that inadequate selection and abuse of antimicrobials use may lead to resistance in various bacteria, and drug resistance in foodborne bacterial enteric pathogens is an almost inevitable consequence of the use of antimicrobial drugs in food-producing animals (Threlfall 2002, McDermott et al. 2018).

The result of the present study showed that the prevelance of *Salmonella* in fecal and litter samples was 46/209 (22%). These findings are also higher than previous studies in China (11.2%) (Zhao et al. 2020), Germany (13.2%) (Zhao et al. 2017a,b), Thailand-Cambodia (35.8%) (Trongjit et al. 2017) and consistent with the investigation performed in Iran (Ghoddusi et al. 2019). Notably, *S.* Infantis accounted for over 82% of broiler strains in the present study. Still, the majority of serovars from poultry sources were *S.* Infantis (33.8%) in Europe (EU) and were higher than % 50 in many EU countries (EFSA 2021). Although, many countries, especially in EU declares that *S.* Typhimurium had a lowest prevelance (0.01%) in broilers, as in our study. Knowledge about the overall occurrence of *Salmonella* serotypes in cattle was varrying as *S.* Montevideo, Typhimurium, Kentucky, Meleagridis, Anatum, Cerro, Mbandaka, Muenster, Newport, and Senftenberg and *S.* Montevideo and *S.* Typhimurium were the two most frequent and dominant serotypes reported (Gutema et al. 2019). Although it was the lowest, we detected *S.* Montevideo from one cow and also *S.* Kitenge was detected in cattle for the first time.

The most common antimicrobial resistance profiles observed were for penicilline (46/46, 100%), lincomycin (43/46, 93.5%), followed by cephalothin 32/46, 69.6%) and streptomycin (31/46, 67.4%). *S.* Infantis represented the most common serovar among *Salmonella* strains isolated from broilers, with 88.4% of *S.* Infantis strains resistant to all antimicrobials tested. Therefore, the distribution of antimicrobial resistance profiles in *S.* Infantis isolated from litter in the present study may be attributed to strains originating from broilers. In this study, the MDR *Salmonella* isolate rate was extremely high as 93.5% and higher than in previous reports (Zhao et al. 2017a,b). During this time, both the prevelance and MDR of *S.* Infantis' in European countries, including Switzerland (Hindermann et al. 2017), Slovenia (Pate et al. 2019), Hungary, Austria, Poland (Nogrady et al. 2012), Israel (Gal-Mor et al. 2010), Germany (García-Soto et al. 2020) and Italy (Franco et al. 2015, Proietti et al. 2020) were increased. Additionally, researchers agreed on acquisition of novel megaplasmid harboured by *S.* Infantis, which confers resistance to multiple drugs in Italy and Israel (Franco et al. 2015, Carfora et al. 2018).

Integrons are important vehicles for *Salmonella* to acquire antimicrobial resistance genes (Bennett 1999) and there seems to exist a strong association between multidrug-resistance and the presence of integrons, a fact that can be easily confirmed when analysing the present results. Similarly, there are several studies focused on investigating the connection between the presence of integrons and resistance genes in multidrug-resistance *Salmonella* strains in different countries (Firoozeh et al. 2011, Asgharpour et al. 2018)

Class 1, 2, 3 integrons were found in high prevelance at 44-85% among *Salmonella* isolates. Interestingly, class 3 were ones with the most higher prevalance. Corresponding proportions among *S.* Infantis were 27 (58.7%) *int*1; 20(43.5%) *int*2; 38 (82.6%) *int*3 in broilers. On the other hand, we found no difference in the distribution of integron types between cattle and broiler isolates. In MDR isolates 65.2% involved class 1; 43.5% were class 2, 82.6% class 3 and both three integrons in 14 (30.4%). MDR encoded by resistance genes clustered in integrons, which are potentially mobile genetic elements, considered to be involved in the transfer of MDR (Asgharpour et al. 2018). Commonly veterinary use antibiotic, namely tetracycline class resistance (both doxycycline, tetracycline and oxytetracycline) was the most common (19.6 %) harboring class 1, 2 and 3 integrons. Class 1 and 2 integrons are commonly observed among MDR isolates, so they are usually referred to as MDR integrons (Antunes et al. 2006, Mazel 2006). While the frequency of class 1 integrons remained stable over time (Asgharpour et al. 2018), our study confirmed a slight increase in the presence of class 2 integrons (42%) in *S.* Infantis isolates. T he results of this study revealed that class 1 and class 2 integrons differ in their behavior as MDR markers, which is similar to the reports of other studies (Dessie et al. 2013, Rahmani et al. 2013). Class 1, 2 and 3 integrons were present in 36%, 42% and 4%, respectively of the MDR isolates in Iran (Asgharpour et al. 2018).

The occurrence of MAR index ranged from 0.2-1.3 and *S.* Infantis isolates with 0.8 had the highest occurrence of 17.4% in this study. The MARI indices (0.2<) in this study confirmed the previous reports that the organisms must have originated from an environment where antibiotics are often used (Chrinius et al. 2014). Thus, from the values of the MARI in this work, it could be asserted that these pathogens might have originated from where these antibiotics are used.

ESBL and/or AmpC producing *Enterobacteriaceae* have been a growing problem throughout the world (Paterson and Bonomo 2005, Livermore et al. 2006). A critical overview of the increasing resistance mecha-

nisms such as active efflux pumps, production of drug-inactivating enzymes, reduced permeability, and modification of the cellular target for drug is also applicable for *Salmonella* (Sefton 2002), and therefore the production of β-lactamases by *Salmonella* is significantly important mechanism that confers the resistance to β-lactam antimicrobials (Revathi et al. 1998, Yan et al. 2003). Resistance to drugs such as quinolones, aminoglycosides, and sulphonamids are common in ESBL-producing bacteria (Hasman et al. 2005, Bush and Jacoby 2010) and also has been considered in combined patterns as NaSSuT and CipNxSSuT with majority restance rate among 65.5-72.9 % in a couple of studies (Hindermann et al. 2017, Pate et al. 2019). Based on the data obtained, nalidixic acid, sulphamethoxazole trimethoprim and gentamicin resistance occured 60.9%, 41.30%, and 15.2% isolates, respectively while amoxicilline clavulanic acid, tazobactam piperacillin, and ampisilin showed low resistance pattern as 8.7%, 10.9% and 13%, respectively in present study.

Of the 46 broiler and cattle-derived *Salmonella* strains isolated in the present study, 6.5% were resistant to cefotaxime. Cefotaxime resistance was observed in *S.* Infantis 4 (8.7%) isolates and in the present study, cefotaxime-resistant *S.* Infantis strains and one *S.* Enteritidis was obtained from litter, and harbored the CTX genes. Of the 46 *Salmonella* isolates, 27 strains (*S.* Infantis, Mbandaka and Typhimurium) from broilers and 1 strain (*S.* Enteritidis) from cattle harbored ESBL genes and CTX and TEM were presented as higher than other genes. In addition, these are consistent with many findings especially for TEM gene (Lu et al. 2011, Aslam et al. 2012). The remaining twenty strains, namely *S.* Infantis harbored TEM, SHV, OXA-1, OXA-2, ACC-1, CMY-1 group, and CMY-2 group. ESBL gene belonging to CTX (high prevalance) were defined as other studies (Franco et al. 2015, Hindermann et al. 2017), TEM, OXA-1, OXA-2, ACC-1, CMY-1 group, CMY-2 group were detected 22(47.8%) in *S.* Infantis and also cephaloitin resistance was common. Third-generation cephalosporin resistance orginating from production of β-lactamase are currently considered a major concern in veterinary medicine (Rhouma and Letellier 2017). In addition, third and fourth generation cephalosporins resistance is associted with CMY-2 gene that produces an p*AmpC*-like β-lactamase (Yan et al. 2003). Unfourtunately, regarding the status, we had no detection on p*AmpC* gene genotypically. In the present study, β-lactamase gene from the CTX was responsible to resistance to cephaloitin (17.4%), cefotaxime and cefixime (6.5%) and cefixime, cefotaxime, ceftazidime (2.2%), although most of the isolates were susceptible to ceftiofur.

Regarding the other antimicrobials, strikingly, results showed that bovine and avian species seemed to be the most relevant source of tetracycline resistance NTS, as compared to other food-related sources. Tetracyclines are commonly used for the treatment of bacterial infections in livestock animals, including tetracycline, doxycycline, chlortetracycline, oxytetracycline, and minocycline (Frech and Schwarz 2000). In this study, the highest antimicrobial resistance patterns were observed in 31 (81.6%) tetracycline, 26 (68.4%) doxycline, 31 (81.6%) oxytetracycline and 26 (68.4%) both of three antibiotic resistant *S.* Infantis isolates from broilers correlates with the recent detection in European countries (Franco et al. 2015, Hindermann et al. 2017, Pate et al. 2019, García-Soto et al. 2020, Proietti et al. 2020) (33.3%) tetracycline resistant of *S.* Enteritidis; 2(66.7) tetracycline resistant of *S.* Kitenge from cattle. Of the 46 *Salmonella* spp. isolates, 12 (26.1%) were determined to show tetracycline resistance; 9 (19.6%) *tet*(B), 3 (6.5%) *tet*(D), 2 (4.3%) *tet*(M) and 1 (2.2%) *tet*(G) and none of the 10 tetracycline resistance genes tested were detected among isolates. However, in *Salmonella* spp. isolates, tetracycline resistance is usually mediated by the following determinants: *tetA, tetB, tetC, tetD* and *tetG* (Michalova et al. 2004, Franco et al. 2015) which correlates well with previous observations (Frech and Schwarz 2000, Hall 2010). Generally, *tet* genes were represented at least one tetracycline resistance profile phenotypically but *S.* Montevideo (that harbored *tetB* gene) were not detected any tetracycline resistance in this study. Incidences of tetracycline resistance have been described recently in Iran and other countries (Michalova et al. 2004, Chuanchuen et al. 2009, Morshed et al. 2010, Franco et al. 2015). However, in *Salmonella* spp. isolates, tetracycline resistance is usually mediated by the following determinants: *tetA, tetB, tetC, tetD* and *tetG* (Michalova et al. 2004, Franco et al. 2015) and several studies reported the range of *Salmonella* carrying the tetracycline resistance gene *tetA* to be 60% to 100% (Chuanchuen et al. 2009, Franco et al. 2015).

Our data suggest that food-producing animals might be simultaneously considered as a reservoir of integrons carrying antibiotic resistance genes especially tetracyclines. Intensive antibiotic resistance over several years was associated with the genetic elements, especially integrons, and also encoded ESBLs. Antibiotic resistance genes may contribute to their spread and niche specificity.

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